

DOCKET NO.: DIBIS-0012US.P1 (Counsel Docket No. 10449)

PATENT

IN THE CLAIMS:

1-26. (cancelled)

27. (currently amended) A method of identifying a pathogen in a biological sample comprising the steps of:

~~selecting a bioagent identifying amplicon;~~

~~selecting a pair of intelligent primers to obtain an amplification product of the bioagent identifying amplicon; and~~

amplifying a plurality of segments of nucleic acid of said pathogen with a plurality of primer pairs to obtain a plurality of amplification products; and

determining the molecular mass of the amplification product, base compositions of at least two members of said plurality of amplification products wherein said molecular mass base compositions identifies the identify said pathogen in the biological said sample.

28. (original) A method of claim 27 wherein the pathogen is a bacterium, a virus, a protozoan, a parasite, a mold, or a fungus.

29. (currently amended) A The method of claim 27 wherein the biological said sample is a biological sample comprising blood, mucus, hair, urine, breath, sputum, saliva, stool, nail, or tissue biopsy.

30. (cancelled)

31. (currently amended) A The method of claim 20 27 wherein the animal is a said sample is obtained from a human.

32. (currently amended) A The method of claim 27 wherein the intelligent primers are said plurality of primer pairs comprises broad range survey primers primer pairs, division-wide primers primer pairs, or drill-down primers primer pairs, or any combination thereof.

33. (currently amended) A The method of claim 27 wherein identification of the said pathogen is accomplished at the genus or species level, and wherein the intelligent primers are said plurality of primer pairs comprises broad range survey primers primer pairs or division-

DOCKET NO.: DIBIS-0012US.P1 (Counsel Docket No. 10449)**PATENT**

wide ~~primers~~ primer pairs.

34. (currently amended) A ~~The~~ method of claim 32 further comprising identifying wherein a subspecies characteristic of the said pathogen ~~is obtained using drill-down primers from the base compositions of at least two drill-down amplification products obtained using a plurality of drill-down primer pairs.~~

35. (currently amended) A ~~The~~ method of claim 34 wherein the said subspecies characteristic is a serotype, a strain type, a sub-strain type, a sub-species type, an emm-type, ~~presence of a bioengineered gene, presence of a toxin gene, presence of an antibiotic resistance gene, presence of a pathogenicity island, or presence of a virulence factor.~~

36. (currently amended) A ~~The~~ method of claim 27 wherein ~~the molecular mass~~ said at least two base compositions are obtained from molecular masses of said plurality of amplification products ~~is~~ determined by mass spectrometry.

37. (currently amended) A ~~The~~ method of claim 36 wherein the said mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FTICR- MS), ion trap mass spectrometry, quadrupole mass spectrometry, magnetic sector mass spectrometry, time of flight (TOF) mass spectrometry, Q-TOF mass spectrometry, or triple quadrupole mass spectrometry.

38. (currently amended) A ~~The~~ method of claim 27 wherein ~~the intelligent primers each member of each primer pair of said plurality of primer pairs are targeted to hybridizes to nucleic acid encoding~~ ribosomal RNA or ~~housekeeping genes~~ a housekeeping gene.

39-49. (cancelled)

50. (new) The method of claim 38 wherein said housekeeping gene encodes a protein that participates in translation, replication, recombination, repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, uptake, secretion, antibiotic resistance, virulence, or pathogenicity.

51. (new) The method of claim 38 wherein said housekeeping gene is a polymerase.

DOCKET NO.: DIBIS-0012US.P1 (Counsel Docket No. 10449)**PATENT**

52. (new) The method of claim 27 wherein said pathogen is a previously unknown pathogen.
53. (new) A method for characterizing a strain type of a pathogen comprising:
amplifying nucleic acid of said pathogen with a plurality of primer pairs that hybridize to a plurality of segments of nucleic acid of said pathogen to obtain a plurality of amplification products, wherein one or more base compositions of said plurality of amplification products differs among different strain types of said pathogen; and
determining base compositions of each member of said plurality of amplification products to obtain a series of base compositions, thereby characterizing said strain type.
54. (new) The method of claim 53 wherein said pathogen is a bacterium, a virus, a protozoan, a parasite, a mold or a fungus.
55. (new) The method of claim 53 wherein said pathogen is obtained from a biological sample.
56. (new) The method of claim 55 wherein said biological sample comprises blood, mucus, hair, urine, breath, sputum, saliva, stool, nail, or tissue.
57. (new) The method of claim 55 wherein said biological sample is obtained from a human.
58. (new) The method of claim 53 wherein said plurality of primer pairs comprises broad range survey primers primer pairs, division-wide primers primer pairs, or drill-down primers primer pairs, or any combination thereof.
59. (new) The method of claim 53 wherein said pathogen comprises a sub-species characteristic.
60. (new) The method of claim 59 wherein said sub-species characteristic is a serotype, a strain type, a sub-strain type, a sub-species type, an emm-type, a bioengineered gene, a toxin gene, an antibiotic resistance gene, a pathogenicity island, or a virulence factor.

DOCKET NO.: DIBIS-0012US.P1 (Counsel Docket No. 10449)**PATENT**

61. (new) The method of claim 53 wherein said base compositions are obtained from molecular masses of said plurality of amplification products determined by mass spectrometry.
62. (new) The method of claim 61 wherein said mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FTICR- MS), ion trap mass spectrometry, quadrupole mass spectrometry, magnetic sector mass spectrometry, time of flight (TOF) mass spectrometry, Q-TOF mass spectrometry, or triple quadrupole mass spectrometry.
63. (new) The method of claim 53 wherein members of said plurality of primer pairs hybridize to nucleic acid encoding ribosomal RNA or housekeeping genes.
64. (new) The method of claim 63 wherein said housekeeping genes encode proteins that participate in translation, replication, recombination, repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, uptake, secretion, antibiotic resistance, virulence, or pathogenicity.
65. (new) The method of claim 63 wherein one or more of said housekeeping genes encodes a polymerase.
66. (new) The method of claim 53 wherein said plurality of primer pairs comprises four or more primer pairs.
67. (new) The method of claim 53 wherein said strain type is a previously unknown strain type.
68. (new) The method of claim 53 further comprising associating said plurality of base compositions with a known strain of said pathogen, thereby identifying the strain type of said pathogen.
69. (new) A method of screening a biological sample to determine the presence or absence of a pathogen comprising:
 contacting nucleic acid of said sample with one or more primer pairs under amplification conditions wherein

DOCKET NO.: DIBIS-0012US.P1 (Counsel Docket No. 10449)**PATENT**

production of one or more amplification products whose base compositions match known base compositions of amplification products of nucleic acid of said pathogen produced with said one or more primer pairs indicates the presence of said pathogen; or

wherein failure to produce one or more amplification products whose base compositions match said known base compositions indicates the absence of said pathogen.

70. (new) The method of claim 69 wherein said pathogen is a bacterium virus, protozoan, parasite, mold, or fungus.

71. (new) The method of claim 69 wherein said biological sample comprises blood or tissue.

72. (new) The method of claim 69 wherein said base compositions are obtained by molecular masses measured by mass spectrometry.

73. (new) The method of claim 72 wherein said mass spectrometry is Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, ion trap mass spectrometry, quadrupole mass spectrometry, magnetic sector mass spectrometry, time-of-flight (TOF) mass spectrometry, Q-TOF mass spectrometry or triple quadrupole mass spectrometry.

74. (new) The method of claim 69 wherein said one or more primer pairs hybridize to nucleic acid encoding ribosomal RNA or housekeeping genes.

75. (new) A method of identifying one or more etiologic agents of disease in a sample comprising the steps of:

amplifying a segment of nucleic acid from said one or more of etiologic agents in said sample with one or more primer pairs to obtain one or more amplification products;

determining base compositions of said one or more amplification products; and

comparing said base compositions with known base compositions of known etiologic agents produced with said one or more primer pairs, thereby identifying said one or more etiologic agents in said sample.

76. (new) The method of claim 75 wherein identification of said one or more etiologic agents is accomplished at the genus or species level, and said one or more primer pairs

DOCKET NO.: DIBIS-0012US.P1 (Counsel Docket No. 10449)**PATENT**

comprise broad range survey primer pairs, division-wide primer pairs or any combination thereof.

77. (new) The method of claim 75 further comprising identifying a subspecies characteristic of said one or more etiologic agents from the base composition of a drill-down amplification product produced with a drill-down primer pair.

78. (new) The method of claim 77 wherein said subspecies characteristic is a serotype, a strain type, a sub-strain type, a sub-species type, an emm-type, a bioengineered gene, a toxin gene, an antibiotic resistance gene, a pathogenicity island, or a virulence factor.

79. (new) The method of claim 75 wherein said base compositions are obtained from molecular masses of said amplification products determined by mass spectrometry.

80. (new) The method of claim 79 wherein said mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FTICR- MS), ion trap mass spectrometry, quadrupole mass spectrometry, magnetic sector mass spectrometry, time of flight (TOF) mass spectrometry, Q-TOF mass spectrometry, or triple quadrupole mass spectrometry.

81. (new) The method of claim 75 wherein said one or more etiologic agents comprise a bacterium, a virus, a protozoan, a parasite, a mold, a fungus, or any combination thereof.

82. (new) The method of claim 75 wherein said sample is a biological sample comprising blood, mucus, hair, urine, breath, sputum, saliva, stool, nail, or tissue.

83. (new) The method of claim 75 wherein said sample is obtained from a human.

84. (new) The method of claim 75 wherein said one or more primer pairs hybridize to nucleic acid encoding ribosomal RNA or housekeeping genes.

85. (new) The method of claim 84 wherein said housekeeping genes encode proteins that participate in translation, replication, recombination, repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, uptake, secretion, antibiotic resistance, virulence, or pathogenicity.

DOCKET NO.: DIBIS-0012US.P1 (Counsel Docket No. 10449)**PATENT**

86. (new) The method of claim 84 wherein one or more of said housekeeping genes encode a polymerase.

87. (new) The method of claim 75 wherein said one or more etiologic agents are previously unknown etiologic agents.